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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/537,562	VENEMA, FOKKE	
	Examiner	Art Unit	
	AMANDA SHAW	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 September 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *24,25,28,29,32,33,36,37,40,41,43,44,47-50,54,55 and 57-64* is/are pending in the application.

4a) Of the above claim(s) *24,25,28,29,32,33,36 and 37* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *40-41, 43-44, 47-50, 54-55, and 57-64* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1)☒ Notice of References Cited (PTO-892)

2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3)☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____

4)☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5)☐ Notice of Informal Patent Application

6)☐ Other: _____.

DETAILED ACTION

1. This action is in response to the papers filed September 27, 2010. Applicant's request for reconsideration of the finality of the rejection of the last Office action is persuasive and, therefore, the finality of that action is withdrawn.

2. Claims 24-25, 28-29, 32-33, 36-37, 40-41, 43-44, 47-50, 54-55, and 57-64 are currently pending.

Claims 24-25, 28-29, 32-33, and 36-37 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected subject matter, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on March 25, 2008.

Withdrawn Rejections

3. The rejection made under 35 USC 102 in section 2 of the Office Action of March 30, 2010 is withdrawn after further consideration.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 40-41, 43-44, 47-50, 54-55, and 57-64 are rejected under 35 U.S.C. 103(a) as being unpatentable over Beckman (US 2003/0134307 Pub 7/2003 and filed 10/2002) in view of Majlessi (Nucleic Acids Research 1998) and Tsourkas (Nucleic Acids Research 2002).

Regarding Claim 40 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5'end), or a MB can consist entirely of 2'-O-methyl nucleotides. (para 0074). Beckman further teaches that the arms of a MB probe that form the stem duplex are about 3-25 nucleotides in length (para 0097).

Regarding Claim 44 Beckman teaches a MB probe wherein the 2'-O-derivatized nucleotides is a 2'-O-methyl-nucleotide (para 0074).

Regarding Claims 54 Beckman teaches a kit comprising primers, polymerase, reagents for performing amplification of an analyte, and a molecular beacon (para 0086).

Beckman does not specifically exemplify a MB probe comprising a stem comprising one or more unmodified nucleotides, and in the 3' strand of the stem, one or more 2'-O-derivatized nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide, and further wherein said probe has better

stability and does not open spontaneously in the presence of contaminants present in an amplification enzyme mixture comprising said MB probe as compared to a MB probe without said stem (clm 40). Beckman does not specifically exemplify a MB probe wherein at least one base pair of said stem contains no nucleotide or nucleotide analogue having an affinity increasing modification (clm 47). Beckman does not specifically exemplify a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification (clm 49).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased Tm which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for Rnase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher Tm and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-

O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified MB probes were well known in the art at the time of the invention.

Accordingly it would have been obvious to one of skill in the art at the time of the invention to have modified the MB probe of Beckman so that the MB comprised a stem comprising one or more unmodified nucleotides, and in the 3' strand of the stem, one or more 2'-O-derivatized nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide. It would have been obvious to make a MB probe wherein at least one base pair of said stem contains no nucleotide or nucleotide analogue having an affinity increasing modification. It would have been obvious to make a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification. In the instant case MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides were known in the art as demonstrated by Beckman. Additionally the advantages of using probes comprising 2'-O-methyl nucleotides were known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe has better stability and does not

open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that stems are 3-25 nucleotides long). A person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. Based on the teachings of Majlessi and Tsourkas an ordinary artisan would have had more than a reasonable expectation of success in designing the claimed MB probes. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Regarding Claim 41 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5'end), or a MB can consist entirely of 2'-O-methyl nucleotides. (para 0074). Beckman further teaches that MB probes typically have a target recognition sequence (loop) of about 7-140 nucleotides in length and arms that form the stem duplex are about 3-25 nucleotides in length (para 0097).

Regarding Claim 43 Beckman teaches a MB probe wherein the one or more nucleotides and/or nucleotide analogues having an affinity increasing modification are 2'-O-derivatized nucleotides (para 0074).

Regarding Claims 55 Beckman teaches a kit comprising primers, polymerase, reagents for performing amplification of an analyte, and a molecular beacon (para 0086).

Beckman does not specifically exemplify a MB probe comprising a stem and a loop wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and staid stem comprises one or more unmodified nucleotides, and in the 3' strand of the stem, one or more 2'-O-derivatized nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide, wherein the sensitivity of said probe to polymorphisms in the target nucleic acid sequence is lowered as compared to a molecular beacon probe without said loop and wherein the spontaneous opening of the probe in the presence of contaminants present in an amplification enzyme mixture comprising said MB probe is lowered as compared to a MB probe without said stem (clm 41). Beckman does not specifically exemplify a MB probe wherein at least one base pair of said stem contains no nucleotide or nucleotide analogue having an affinity increasing modification (clm 48). Beckman does not specifically exemplify a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification (clm 50).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased Tm which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy

oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for Rnase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher Tm and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified MB probes were well known in the art at the time of the invention.

Accordingly it would have been obvious to one of skill in the art at the time of the invention to have modified the MB probe of Beckman so that the MB comprised a stem and a loop wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and staid stem comprises one or more unmodified nucleotides, and in the 3' strand of the stem, one or more 2'-O-derivatized nucleotides. It would have been obvious to make a MB probe wherein at least one base pair of said stem contains no

nucleotide or nucleotide analogue having an affinity increasing modification. It would have been obvious to make a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification. In the instant case MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides were known in the art as demonstrated by Beckman. Additionally the advantages of using probes comprising 2'-O-methyl nucleotides were known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe is less likely to open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that stems are 3-25 nucleotides long). Additionally determining the optimum placement of the 2'-O-methyl nucleotides in the loop region so that the sensitivity of the probe to polymorphisms in the target nucleic acid sequence is lowered is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the loop region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that the loop region can be as small as 7 nucleotides long). A person of ordinary skill has good

reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. Based on the teachings of Majlessi and Tsourkas an ordinary artisan would have had more than a reasonable expectation of success in designing the claimed MB probes. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Regarding Claim 57 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5'end), or a MB can consist entirely of 2'-O-methyl nucleotides. (para 0074). Beckman further teaches that the arms of a MB probe that form the stem duplex are about 3-25 nucleotides in length (para 0097).

Regarding Claim 58 Beckman teaches a MB probe wherein the 2'-O-derivatized nucleotide is a 2'-O-methyl nucleotide (para 0074).

Beckman does not specifically exemplify a MB probe comprising a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, wherein said one or more nucleotides or nucleotide analogues is selected from the group consisting of a 2'-O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2'O-derivatized nucleotide and only

one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification, and further wherein said probe has better stability and does not open spontaneously in the presence of contaminants present in an amplification enzyme mixture comprising said MB probe as compared to a MB probe without said stem (clm 57).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased Tm which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for Rnase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher Tm and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly

higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified MB probes were well known in the art at the time of the invention.

Accordingly it would have been obvious to one of skill in the art at the time of the invention to have modified the MB probe of Beckman so that the MB comprised a stem comprising one or more nucleotides or nucleotide analogues having an affinity increasing modification, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2'O-derivatized nucleotide and only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification. In the instant case MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides were known in the art as demonstrated by Beckman. Additionally the advantages of using probes comprising 2'-O-methyl nucleotides were known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe has better stability and does not open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that stems are 3-25 nucleotides long). A person of ordinary skill has good reason to pursue the known options within his

or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. Based on the teachings of Majlessi and Tsourkas an ordinary artisan would have had more than a reasonable expectation of success in designing the claimed MB probes. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Regarding Claim 59 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5'end), or a MB can consist entirely of 2'-O-methyl nucleotides. (para 0074). Beckman further teaches that MB probes typically have a target recognition sequence (loop) of about 7-140 nucleotides in length and arms that form the stem duplex are about 3-25 nucleotides in length (para 0097).

Beckman does not specifically exemplify a MB probe comprising a stem and a loop wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and staid stem comprises one or more 2'-O-methyl nucleotides and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide and only one base pair of said stem comprises no nucleotides or nucleotide analogue having an affinity increasing modification, wherein the sensitivity of said probe to polymorphisms in the target nucleic acid sequence is

lowered as compared to a molecular beacon probe without said loop and wherein the spontaneous opening of the probe in the presence of contaminants present in an amplification enzyme mixture comprising said MB probe is lowered as compared to a MB probe without said stem (clm 59).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased Tm which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for Rnase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher Tm and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly

higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified MB probes were well known in the art at the time of the invention.

Accordingly it would have been obvious to one of skill in the art at the time of the invention to have modified the MB probe of Beckman so that the MB comprised a stem and a loop wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and staid stem comprises one or more 2'-O-methyl nucleotides, and one or more unmodified nucleotides, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide and only one base pair of said stem comprises no nucleotides or nucleotide analogue having an affinity increasing modification. In the instant case MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides were known in the art as demonstrated by Beckman. Additionally the advantages of using probes comprising 2'-O-methyl nucleotides were known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe is less likely to open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that

stems are 3-25 nucleotides long). Additionally determining the optimum placement of the 2'-O-methyl nucleotides in the loop region so that the sensitivity of the probe to polymorphisms in the target nucleic acid sequence is lowered is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the loop region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that the loop region can be as small as 7 nucleotides long). A person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. Based on the teachings of Majlessi and Tsourkas an ordinary artisan would have had more than a reasonable expectation of success in designing the claimed MB probes. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Regarding Claim 60 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5'end), or a MB can consist entirely of 2'-O-methyl nucleotides. (para 0074). Beckman further teaches that the arms of a MB probe that form the stem duplex are about 3-25 nucleotides in length (para 0097).

Regarding Claim 61 Beckman teaches a MB probe wherein the 2'-O-derivatized nucleotides is a 2'-O-methyl-nucleotide (para 0074).

Beckman does not specifically exemplify a MB probe comprising a stem comprising one or more unmodified nucleotides, and in each strand of said stem, at least one nucleotide or nucleotide analogue having an affinity increase modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2'-O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide, and further wherein said probe has better stability and does not open spontaneously in the presence of contaminants present in an amplification enzyme mixture comprising said MB probe as compared to a MB probe without said stem (clm 60). Beckman does not specifically exemplify a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification (clm 62).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased Tm which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for Rnase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher Tm and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified MB probes were well known in the art at the time of the invention.

Accordingly it would have been obvious to one of skill in the art at the time of the invention to have modified the MB probe of Beckman so that the MB comprised a stem comprising one or more unmodified nucleotides, and in each strand of said stem, at least one nucleotide or nucleotide analogue having an affinity increase modification, wherein said one or more nucleotides or nucleotide analogues are selected from the group consisting of a 2'-O-derivatized nucleotide, a locked nucleic acid, and a peptide nucleic acid, wherein each base pair of said stem comprises no more than one 2'-O-derivatized nucleotide. It would have been obvious to make a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification. In the instant case MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides were known in the art as demonstrated by Beckman. Additionally the advantages of using probes comprising 2'-

O-methyl nucleotides were known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe has better stability and does not open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that stems are 3-25 nucleotides long). A person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. Based on the teachings of Majlessi and Tsourkas an ordinary artisan would have had more than a reasonable expectation of success in designing the claimed MB probes. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Regarding Claim 63 Beckman teaches molecular beacon (MB) probes comprising modified nucleotides. Specifically Beckman teaches that a MB probe comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5'end), or a MB can consist entirely of 2'-O-methyl nucleotides.

(para 0074). Beckman further teaches that MB probes typically have a target recognition sequence (loop) of about 7-140 nucleotides in length and arms that form the stem duplex are about 3-25 nucleotides in length (para 0097).

Beckman does not specifically exemplify a MB probe comprising a stem and a loop wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and said stem comprises one or more unmodified nucleotides, and in each strand of said stem, at least one 2'-O-methyl nucleotide, wherein each base pair of said stem comprises no more than one 2'-O-methyl nucleotide, wherein the sensitivity of said probe to polymorphisms in the target nucleic acid sequence is lowered as compared to a molecular beacon probe without said loop and wherein the spontaneous opening of the probe in the presence of contaminants present in an amplification enzyme mixture comprising said MB probe is lowered as compared to a MB probe without said stem (clm 63). Beckman does not specifically exemplify a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification (clm 64).

However Majlessi teaches that 2'-O-methyl oligoribonucleotide probes afford multiple advantages over 2' deoxy oligoribonucleotide probes for detecting RNA targets, including greatly increased Tm which allows use of shorter probes, faster kinetics of hybridization, ability to bind to structured targets under conditions where 2' deoxy oligoribonucleotide probes will not and significantly improved specificity. Majlessi further states that these advantages render 2'-O-methyl oligoribonucleotide probes

superior to 2' deoxy oligoribonucleotide probes for use in assays that detect RNA targets (page 2224 and 2229). Thus the benefits of using 2'-O-methyl modified probes were well known in the art at the time of the invention.

Additionally Tsourkas teaches that 2'-O-methyl oligoribonucleotides bind RNA with higher affinity and faster kinetic hybridization rates, resist nuclease degradation, and do not form a substrate for Rnase H. Tsourkas further teaches that 2'-O-methyl MB probes form a more stable stem-loop structure because of the presence of the 2'-O-methyl nucleotides. In the absence of target, the 2'-O-methyl MB exhibited a higher Tm and a lower level of background fluorescence compared with the 2' deoxy MB. The 2'-O-methyl modification of the MB backbone resulted in a higher affinity for target mRNA. The melting temperature of the 2'-O-methyl/RNA hybrid was found to be significantly higher than that of the 2'-deoxy/RNA hybrid (page 5173). Thus the benefits of using 2'-O-methyl modified MB probes were well known in the art at the time of the invention.

Accordingly it would have been obvious to one of skill in the art at the time of the invention to have modified the MB probe of Beckman so that the MB comprised a stem and a loop wherein said loop comprises: one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and one or more unmodified nucleotides; and said stem comprises one or more unmodified nucleotides, and in each strand of said stem, at least one 2'-O-derivatized nucleotide, wherein each base pair of said stem comprises no more than one 2'-O-methyl nucleotide. It would have been obvious to make a MB probe wherein only one base pair of said stem comprises no nucleotide or nucleotide analogue having an affinity increasing modification. In the

instant case MB probes comprising standard deoxyribonucleotides and one or more 2'-O-methyl nucleotides were known in the art as demonstrated by Beckman. Additionally the advantages of using probes comprising 2'-O-methyl nucleotides were known in the prior art and are taught by Majlessi and Tsourkas. Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe is less likely to open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that stems are 3-25 nucleotides long). Additionally determining the optimum placement of the 2'-O-methyl nucleotides in the loop region so that the sensitivity of the probe to polymorphisms in the target nucleic acid sequence is lowered is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the loop region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that the loop region can be as small as 7 nucleotides long). A person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. Based on the teachings of Majlessi and Tsourkas an ordinary artisan would have had more than a

reasonable expectation of success in designing the claimed MB probes. Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Response To Arguments

5. In the response filed September 27, 2010, the Applicants traversed the rejections made under 35 USC 102 and 35 USC 103.

Regarding the rejection made under 35 USC 102 the Applicants state that Beckman discloses a MB comprising standard deoxyribonucleotides can also comprise one or more 2'-O-methyl nucleotides (e.g., at its 5' end), or a MB can consist entirely of 2'-O-methyl nucleotides (para 0074). The Applicants state that while it is not clear which of the three 5' ends of the MB is being referred to in this sentence, i.e., the first or second arms or the loop, it is clear that the references is to a 5' end and not a 3' end. The Applicants state that Beckman is trying to solve the problem of reducing the nuclease degradation (specifically 5' to 3' nuclease activity) of MB probes that are hybridized to a target nucleic acid and the solution provided is nuclease resistant MBs produced by either by replacing unmodified nucleotides entirely with modified nucleotides or with only one or more modified nucleotides at the 3'end.

These arguments have been fully considered and are persuasive. The rejections made under 35 USC 102 have been withdrawn and new art rejections made under 35 USC 103 have been set forth in the Office Action.

Regarding the rejections made under 35 USC 103 the Applicants argue that

Beckman fails to teach or suggest a MB probe having modified nucleotides in the 3' strand of the stem. Further they argue that Beckman fails to teach or suggest a MB probe comprising a stem having no base pair with more than one modified nucleotide and only one base pair with no modified nucleotide or a stem wherein each base pair comprises no more than one 2'-O-methyl nucleotides and only one base pair of the stem comprises no modified nucleotides or a stem wherein each strand of the stem comprises at least one modified nucleotide and each base pair of the stem comprises no more than one 2'-O-methyl nucleotide as claimed in the present invention.

These arguments have been fully considered and it is noted that the rejection acknowledges that Beckman does not teach these limitations.

The Applicants argue that the secondary references, Majlessi et al. and Tsourkas et al., both describes the advantages of probes made entirely of modified nucleotides as compared to probes made entirely of unmodified nucleotides. The Applicants state that these references provide no guidance to one of ordinary skill in the art to specifically select the MB probes as claimed. The Applicants note that Majlessi et al. does not teach MB probes but rather teaches linear probes comprised entirely of 2'-O-methyl nucleotides. The Applicants state that Majlessi et al. describes the advantages of these linear probes only in relationship to the binding of the probe to the target DNA/RNA. The Applicants assert that the higher Tms, affinities, and hybridization kinetics are related only to hybridization of the probes to the target nucleic acid and not to hybridization between two strands (5' and 3' ends) of MB probes. The Applicants state that Tsourkas et al. describes the advantages of probes comprised entirely of 2'-O-methyl

nucleotides as compared with probes comprised entirely of 2'- deoxynucleotides. The Applicants note that Tsourkas et al. discloses that the stem-loop structure of a MB probe comprised entirely of 2'-O-methyl nucleotides is more stable than a MB probe comprised entirely of 2'- deoxynucleotides (page 5169, first full paragraph and page 5170, second column, first full paragraph). Applicants assert that in order to achieve a stable MB probe as taught by Tsoukas et al., at least the stem of the MB probe must consist entirely of 2'-O-methyl nucleotides so that when the stem is hybridized to itself (forming the hairpin structure), 2-O-methyl nucleotides are able to hybridize with other 2'-O-methyl nucleotides. Applicants then argue that this is a teaching away of using MB probes having 2'-O-methyl nucleotides hybridized only to unmodified nucleotides and/or at least one base pair of the stem comprising no modified nucleotides as claimed in the present invention.

These arguments have been fully considered. The Applicants are correct in that Majlessi and Tsourkas both describe the advantages of probes made entirely of modified nucleotides as compared to probes made entirely of unmodified nucleotides. However this does not mean that the teachings of Majlessi and Tsourkas are not relevant to the claimed invention. The secondary references have been cited to demonstrate that it was well known in the art at the time of the invention that there were advantages to using linear probes and MB probes comprising 2'-O-methyl oligonucleotides. Majlessi refers to linear probes and teaches that 2'o-methyl oligoribonucleotide probes bound to RNA targets faster and with much higher melting temperatures than corresponding 2'-deoxyribonucleotide probes (abstract). This

teaching is relevant to the instant claims because it describes the base pairing of a 2'-o-methyl nucleotide and an unmodified nucleotide which is required by the present claims since each base pair in the stem comprises no more than one 2'-o-methyl nucleotides. Tsourkas refers to MB probes and teaches that "We found that the 2-o-methyl molecular beacons hybridize to RNA more quickly and with higher affinity than 2'-deoxy molecular beacons even though they exhibit a much more stable stem-loop structure" (page 5169, col 1). Although Majlessi and Tsourkas compared probes consisting of 2'-O-methyl oligoribonucleotides to probes consisting of 2' deoxy oligoribonucleotides one of skill in the art would have recognized that probes consisting of both 2'-O-methyl nucleotides and 2' deoxy oligoribonucleotides would also have some advantageous properties. Further the argument that Tsourkas teaches away from the claimed invention is misleading. Tsourkas states that 2'-O-methyl MB form a more stable stem loop structure because of the 2'-O-methyl/2'-O-methyl interaction. This statement is in comparison to MB consisting of only 2'-deoxy nucleotides. Tsourkas does not make any comment on the stability of a stem loop structure where 2'-O-methyls interact with 2'-deoxy nucleotides as in the claimed invention.

The Applicants further argue that the Examiner has not provided any reasoned explanation as to how making the probes of the present invention would be routine experimentation other than to suggest that an ordinary artisan would have had more than a reasonable expectation of success in designing probes that have better stability and do not open spontaneously through the use of a computer program. No specific

computer program is identified and applicants are unaware of any computer program that was available at the time of the filing of the present invention.

This argument has been fully considered but is not persuasive. Determining the optimum placement of the 2'-O-methyl nucleotides in the stem region so that the probe is less likely to open spontaneously is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the stem region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that stems are 3-25 nucleotides long and the MB probes of the specification have stems that are 6-7 nucleotides long). Additionally determining the optimum placement of the 2'-O-methyl nucleotides in the loop region so that the sensitivity of the probe to polymorphisms in the target nucleic acid sequence is lowered is considered to be routine experimentation. It is obvious to try different placements of the 2'-O-methyl nucleotides in the loop region, particularly since there are only a limited number of positions within the stem region (Beckman teaches that the loop region can be as small as 7 nucleotides long and the MB probes of the specification have loops that are 12-14 nucleotides long). In theory this could be done by making a collection of MB probes with different stem sequences and then testing each one for effectiveness. Additionally computer programs aid in the selection of molecular beacon probes were well known in the art at the time of the invention. Specifically Goforth (The Scientist Vol 15 Issue 11 page 21 5/28/2001) teaches that the software designers at Premier Biosoft released Beacon Designer ™ which is a tool for the design of molecular beacons. The programs adjusts beacon T_m by selecting a stem of appropriate length, but in order to

design effective beacons, the search and stability parameters default to published optimal values. As such routine experimentation could be used to design the probes of the present invention.

The Applicants argue that the ordinary skilled artisan would have to have chosen from an extremely large number of possible configurations for MBs in order to identify any particular MB that would solve the problem of stability and spontaneous opening. Beckman discloses MBs comprised entirely of modified nucleotides or comprised of one or more modified nucleotides in the 5' end of the MB; however, fails to disclose the specific MBs as claimed in the present invention. The secondary references only discuss the advantages of probes (MBs and linear) that are comprised entirely of modified nucleotides over those that are comprised entirely of unmodified nucleotides. Simply disclosing the advantages of MBs comprised entirely of modified nucleotides does not provide the guidance one of ordinary skill in the art would need in order to have a reasonable expectation of achieving the presently claimed invention. Based on what was known at the time the present application was filed, one of ordinary skill in the art would not have been able to predict which of the many possible MBs would have greater stability and lower spontaneous opening and consequently, would have had no reasonable expectation of success in achieving the presently claimed invention.

This argument has been fully considered but is not persuasive. The MB probes exemplified in the specification are only 26 bp long. To make the MB probe of claim 40, with a stem comprising a single 2'-O-methyl nucleotide there is only a small number of places where the modified nucleotide can go. While one may not have been able to

predict which MBs would have greater stability and lower spontaneous opening, through routine experimentation this could have easily been determined. As such one of skill in the art would have had a reasonable expectation of achieving the presently claimed invention.

Finally, the Applicants argue that they have unexpectedly discovered that the designing of a MB probe that has better stability and that does not open spontaneously, depends both on the presence and position of the nucleotide analogues in the stem and whether the nucleotide analogues are base-paired with other nucleotide analogues or with unmodified nucleotides. See, for example, Table 6 of Example 4 of the present specification, which shows that the use of MB probes consisting entirely of base pairs having only one type of nucleotide (unmodified or 2'-O-methyl nucleotides) results in high levels of spontaneous opening of the probe. Notably, the MB4 probe having all modified nucleotides has a greater percentage of spontaneous opening (IBL-Increase of Baseline) than Reference MB, which is comprised entirely of unmodified nucleotides. The MB4 probe also has a greater percentage of spontaneous opening as compared to MB probes comprising a combination of unmodified and modified nucleotides. MB probes having 2'-O-methyl nucleotides base-paired with unmodified nucleotides also show increased stability, which is surprising in view of what was known in the art at the time the present invention was made. See Tsourkas et al., page 5173, first column, last sentence (teaching that the greater stability of the stem-loop structure of the MB probes is the result of the 2'-O-methyl/2'-O-methyl interactions). Furthermore, as demonstrated with probes MB8 and MB9 (Figures 17 and 18, respectively), having one base pair in

the stem of the MB that is comprised of unmodified nucleotides, results in an unexpectedly low level of spontaneous opening as compared with probes not having such structure (see, Example 4, Table 6, and Figures 17 and 18). None of the cited art teaches or suggests that the content and placement of the modified nucleotides in a MB probe with respect to unmodified nucleotides could or would play a role in the functional features of a MB probe.

This argument has been fully considered but is not persuasive. In the instant case one of skill in the art would have certainly recognized that designing a MB probe that has better stability and that does not open spontaneously depends on both the presence and position of the nucleotide analogues in the stem and whether the nucleotide analogues are base-paired with other nucleotide analogues or with unmodified nucleotides. Referring to Table 6, MB4 probe having all modified nucleotides a 9% chance of spontaneous opening and the reference MB probe having all unmodified nucleotides has a 7% chance of opening up. Therefore it appears that the probe having all modified nucleotides has a slightly higher chance of opening up than the probe having all unmodified nucleotides however its not clear if 7% vs. 9% is considered to be significantly higher chance of opening up. Table 6 also shows that the MB4 (9%) has a greater percentage of spontaneous opening as compared to MB probes comprising a combination of unmodified and modified nucleotides. Here it is only possible to compare MB4 (9%) with MB1 (6%), MB2 (5%), and MB3 (3%). Again its not clear if these differences (9% vs. 6%, 9% vs. 5%, and 9% vs. 3%) are considered to be significant differences. It is not possible to compare MB4 with MB5-MB9 because

those probes have a different number of nucleotides in the stem. The Applicants argument that MB probes having 2'-O-methyl nucleotides base-paired with unmodified nucleotides show increased stability is misleading because MB10 shows a MB probe having 2'-O-methyl nucleotides base-paired with unmodified nucleotides, and this particular MB had the highest chance of opening up (10%). Table 6 shows that probes MB8 and MB9, having one base pair in the stem of the MB that is comprised of unmodified nucleotides, results in a low level of spontaneous opening (1.5% and 0.5%). It is noted that MB8 (1.5%) can only be compared to MB5 (5%), MB6 (4%), and MB7 (5%). Here it is not clear if these differences (1.5% vs. 5%, 1.5% vs. 4%, and 1.5% vs. 5%) are considered to be significant differences. Additionally MB9 can not be compared to any of the other probes since it has more nucleotides in the stem region than all of the other probes. Further it is noted that the instant claims are not limited to MB probes having one base pair in the stem that is comprised of unmodified nucleotides. The claims recite that the stem comprises one or more unmodified nucleotides which encompasses having multiple bases pairs in the stem that are comprised of unmodified nucleotides. The Applicants have not demonstrated that having >3 base pairs of unmodified nucleotides in the stem would also result in lower levels of spontaneous opening. For these reasons the Applicants arguments are not persuasive.

Conclusion

6. No Claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amanda M. Shaw whose telephone number is (571)

272-8668. The examiner can normally be reached on Mon-Fri 7:30 TO 4:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached at 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Examiner 1634

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